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Supplemental Material

Integrated Studies on Male Reproductive Toxicity of Decabromodiphenyl Ethane in Zebrafish Spermatozoa *Ex Vivo*, Male Zebrafish *in Vivo*, and GC-1 Cells *in Vitro*

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Zhixiang Xu, Xinxin Ren, Biran Zhu, Yanna Han, Yongyong Guo, Jian Han, and
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Table of Contents

Figure S1. Effects of *ex vivo* exposure of DBDPE on zebrafish spermatozoa motility in the pre-experiment. (A) Total motility (TM, %) of spermatozoa. (B) Progressive motility (PM, %) of spermatozoa. (C) Average path velocity (VAP, µm/s) of spermatozoa. (D) Straight line velocity (VSL, µm/s) of spermatozoa. (E) Curvilinear velocity (VCL, µm/s) of spermatozoa. (F) Linearity (LIN, %) of spermatozoa. Data represented as the means ± standard error of the mean (SEM) (*n* = 33-36). $*P < 0.05$ and $**P < 0.01$ indicate significant differences between the exposure groups and the control group by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test. “ns” indicates no significant difference between blank control and solvent control (0.05% DMSO) groups. Data are reported in Excel Table S10.

Figure S2. Effects of *ex vivo* exposure to DBDPE on zebrafish spermatozoa motility and hatching rate of embryos by by artificial fertilization. (A) Average path velocity (VAP, µm/s) of zebrafish spermatozoa. (B) Straight line velocity (VSL, µm/s) of zebrafish spermatozoa. (C) Curvilinear velocity (VCL, µm/s) of zebrafish spermatozoa (*n* = 30-41). (D) Hatching rate determined at 72 h post hatching of zebrafish embryos derived by artificial fertilization (*n* = 9). Data represented as the means ± standard error of the mean (SEM). No statistical significance was observed in all the DBDPE-exposed group compared to the control, by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test. Data are reported in Excel Table S11.

Figure S3. Effects of DBDPE in vivo exposure on different parameters of reproductive behavior in male zebrafish. (A) Cumulative mating duration. (B) Mating rate. (C) Mating frequency. (D) Cumulative body contact duration. (E) Body contact rate. (F) Body contact frequency. n = 19-21. Data represented as the means ± standard error of the mean (SEM). No statistical significance was observed in all the DBDPE-exposed group compared to the control, by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test. Data are reported in Excel Table S12.

Figure S4. Area ratio of interstitial space/whole testicular section in male zebrafish testes after 2-month *in vivo* exposure to DBDPE. Data represented as the means ± standard error of the mean (SEM), n = 7-9. No statistical significance was observed in all the DBDPE-exposed group compared to the control, by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test. P = 0.843, 0.149, 0.056, 0.116 in 0.1, 1, 10,100 nM DBDPE exposure group, respectively. See Excel Table S13 for summary data.

Figure S5. Whole proteome and phosphoproteome male in male zebrafish testes after exposure to 100 nM DBDPE. (A) Distribution of the phosphorylated serine, threonine, and tyrosine residues among the identified phosphorylation sites in the phosphoproteome. (B) Numbers and percentages of up-regulated and down-regulated proteins, and those that did not significantly differ in 100 nM DBDPE treated zebrafish testes relative to the control. (C) Numbers and percentages of up-regulated and down-regulated phospho-protiens and those that did not significantly differ in 100 nM DBDPE treated zebrafish testes relative to the control. (D) GO enrichment analysis of DEPs. (E) GO enrichment analysis of proteins harboring differentially phosphorylated sites. See Excel Table S14 for summary data.

Figure S6. The relative mRNA expression levels of target genes in testes of zebrafish exposed to lower concentrations of DBDPE (0, 0.1, 1, 10 nM) for two months (n =3 in each group). Data were represented as the means ± standard error of the mean (SEM). $*P < 0.05$, $**P < 0.01$ and $***P < 0.01$indicate significant differences between the exposure groups and the control group by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test. Red and blue colors indicate upregulation and downregulation of target genes in exposed zebrafish testes relative to control, repectively. Color intensity is proportional to $\log_2$(fold change). The values in each box indicate $\log_2$(fold change) of mRNA expression levels of target genes relative to control. See Excel Table S15 for summary data.

Figure S7. Primary investigation on the effects of DBDPE on MMP and ATP contents in GC-1 cells by setting a series of concentrations *in vitro*. (A) Mitochondrial membrane potential (MMP) of GC-1 cells following exposure to 0.01-10 μM DBDPE for 48 h (n = 4-5). (B) ATP contents of GC-1 cells following exposure to 0.01-10 μM DBDPE for 48 h (n = 3). Data were represented as the means ± standard error of the mean (SEM). $*P < 0.05$ indicates significant differences between the exposure groups and the control group. Data are reported in Excel Table S10.

Figure S8. Correlation analysis of the investigated parameters in GC-1 cells between the 1 uM DBDPE exposure group and the oxamate exposure group. For most of the investigated parameters were significantly changed in 1 μM DBDPE exposure group and oxamate exposure group, with similar change trends observed. A pearson correlation analysis were conducted to further figure out their changing correlationship. *"R"* and *"P"* indicate the correlation coefficient and the significant difference, respectively; their values are shown in the figure. Red and blue colour represent positive and negative correlation, respectively.

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Table S2. CASA input parameters for zebrafish.

Table S3. Detailed informations of primers for qRT-PCR used in the present study.

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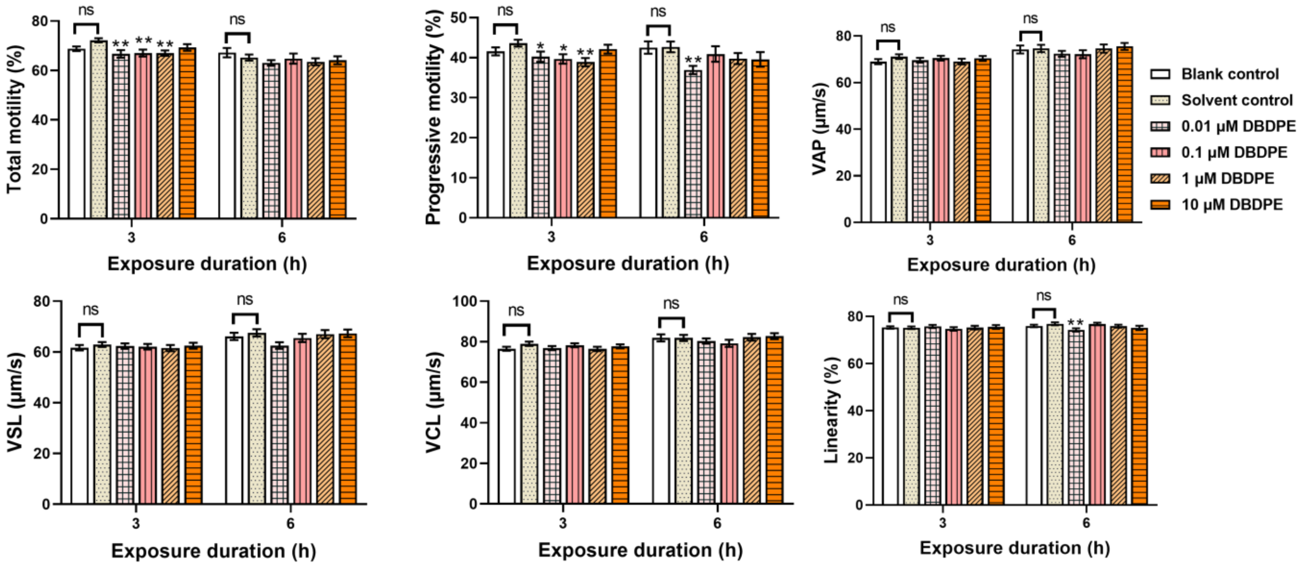
Table S5. Basic developmental parameters of exposed male fish and F1 offspring larvae.

Additional File- Excel Document

Supplementary figures



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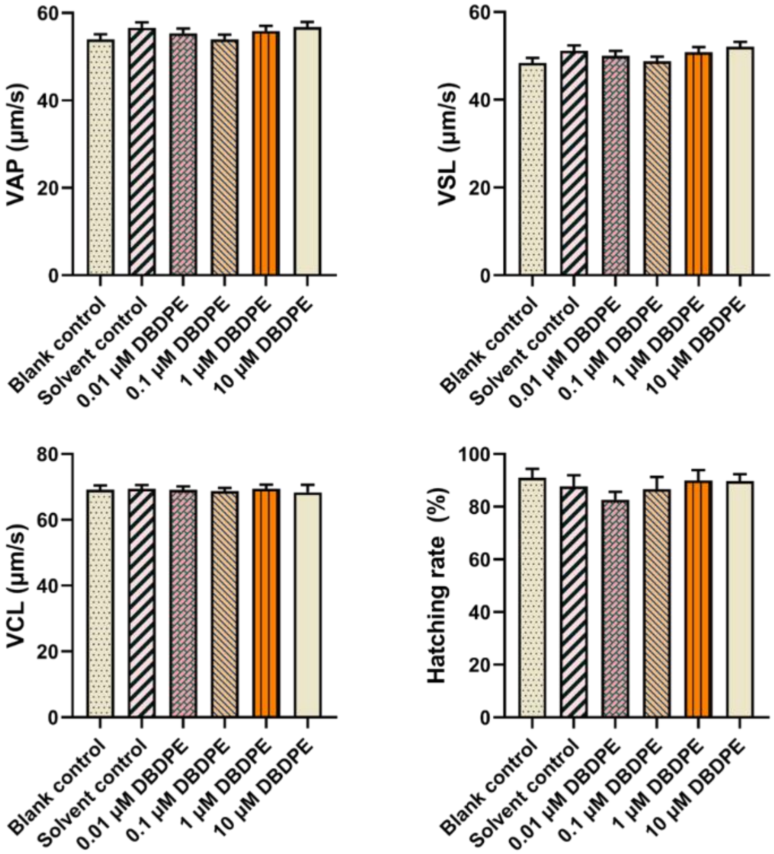

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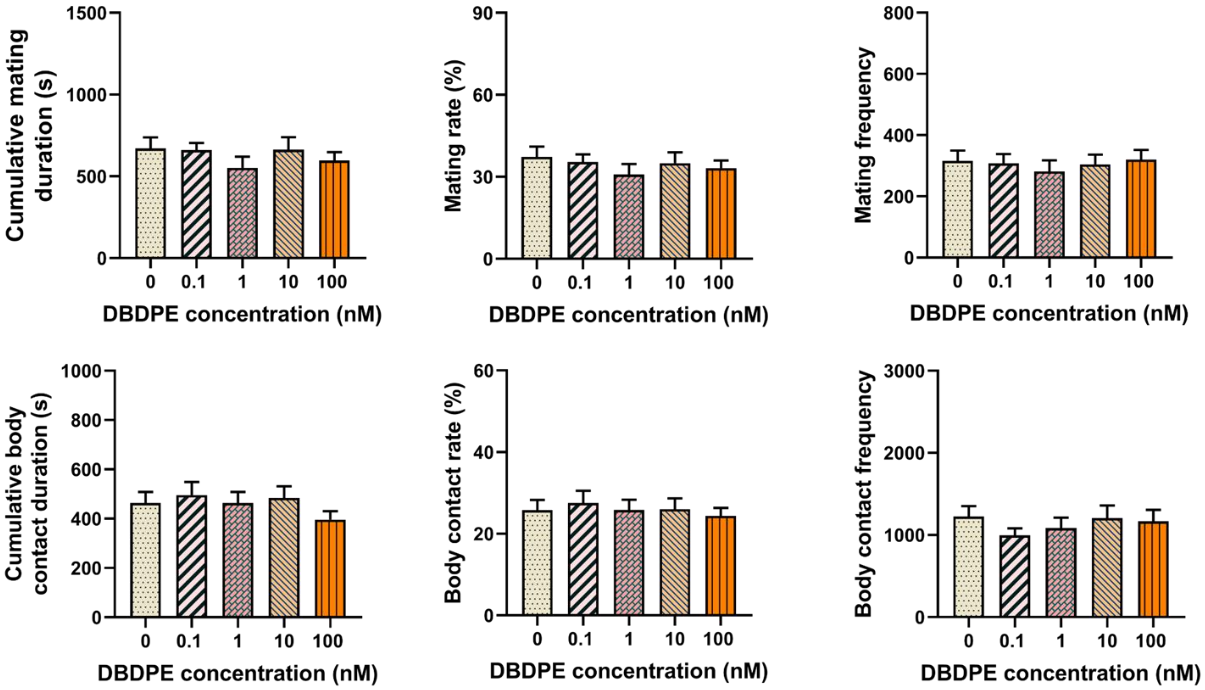

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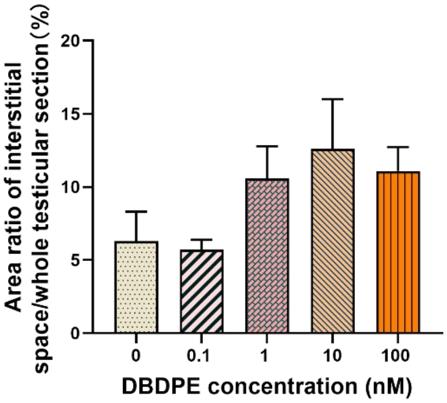

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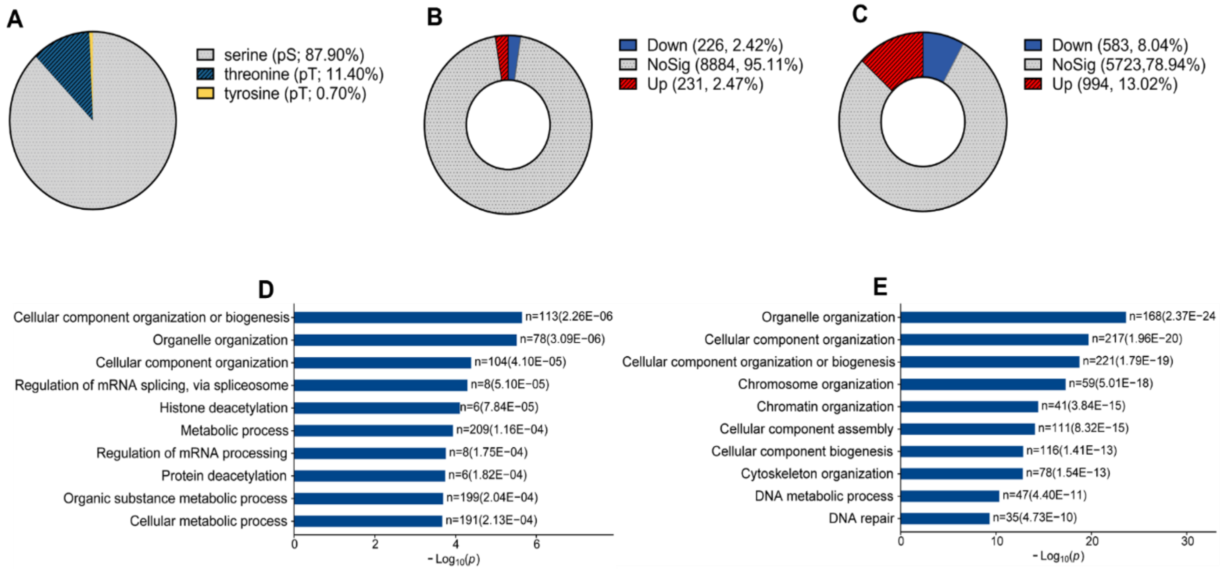

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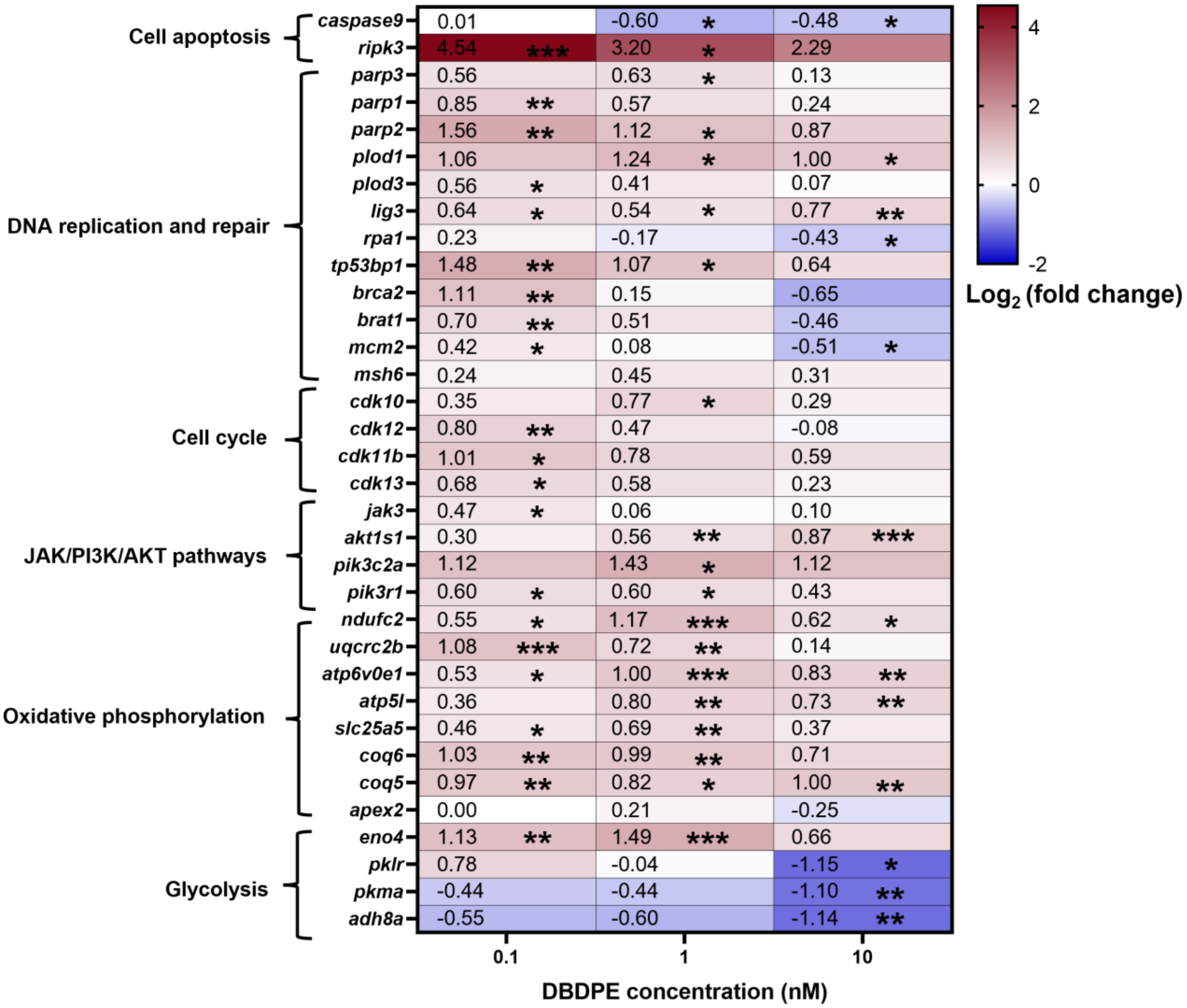
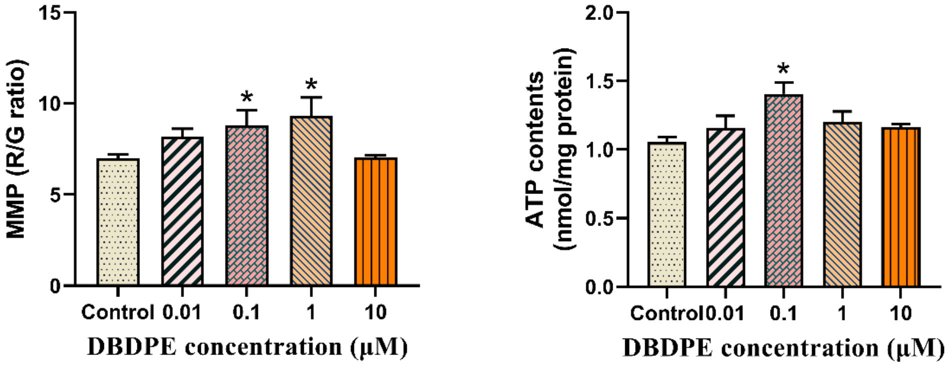


		0.1	1	10
Cell apoptosis	*caspase9*	0.01	-0.60 *	-0.48 *
	ripk3	4.54 ***	3.20 *	2.29
DNA replication and repair	*parp3*	0.56	0.63 *	0.13
	parp1	0.85 **	0.57	0.24
	parp2	1.56 **	1.12 *	0.87
	plod1	1.06	1.24 *	1.00 *
	plod3	0.56 *	0.41	0.07
	lig3	0.64 *	0.54 *	0.77 **
	rpa1	0.23	-0.17	-0.43 *
	tp53bp1	1.48 **	1.07 *	0.64
	brca2	1.11 **	0.15	-0.65
	brat1	0.70 **	0.51	-0.46
	mcm2	0.42 *	0.08	-0.51 *
	msh6	0.24	0.45	0.31
Cell cycle	*cdk10*	0.35	0.77 *	0.29
	cdk12	0.80 **	0.47	-0.08
	cdk11b	1.01 *	0.78	0.59
	cdk13	0.68 *	0.58	0.23
JAK/PI3K/AKT pathways	*jak3*	0.47 *	0.06	0.10
	akt1s1	0.30	0.56 **	0.87 ***
	pik3c2a	1.12	1.43 *	1.12
	pik3r1	0.60 *	0.60 *	0.43
Oxidative phosphorylation	*ndufc2*	0.55 *	1.17 ***	0.62 *
	uqcrc2b	1.08 ***	0.72 **	0.14
	atp6v0e1	0.53 *	1.00 ***	0.83 **
	atp5l	0.36	0.80 **	0.73 **
	slc25a5	0.46 *	0.69 **	0.37
	coq6	1.03 **	0.99 **	0.71
	coq5	0.97 **	0.82 *	1.00 **
	apex2	0.00	0.21	-0.25
Glycolysis	*eno4*	1.13 **	1.49 ***	0.66
	pklr	0.78	-0.04	-1.15 *
	pkma	-0.44	-0.44	-1.10 **
	adh8a	-0.55	-0.60	-1.14 **

Figure S6. The relative mRNA expression levels of target genes in testes of zebrafish exposed to lower concentrations of DBDPE (0, 0.1, 1, 10 nM) for two months (n =3 in each group). Data were represented as the means ± standard error of the mean (SEM). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.01$indicate significant differences between the exposure groups and the control group by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test. Red and blue colors indicate upregulation and downregulation of target genes in exposed zebrafish testes relative to control, repectively. Color intensity is proportional to log$_2$(fold change). The values in each box indicate log$_2$(fold change) of mRNA expression levels of target genes relative to control. See Excel Table S15 for summary data.



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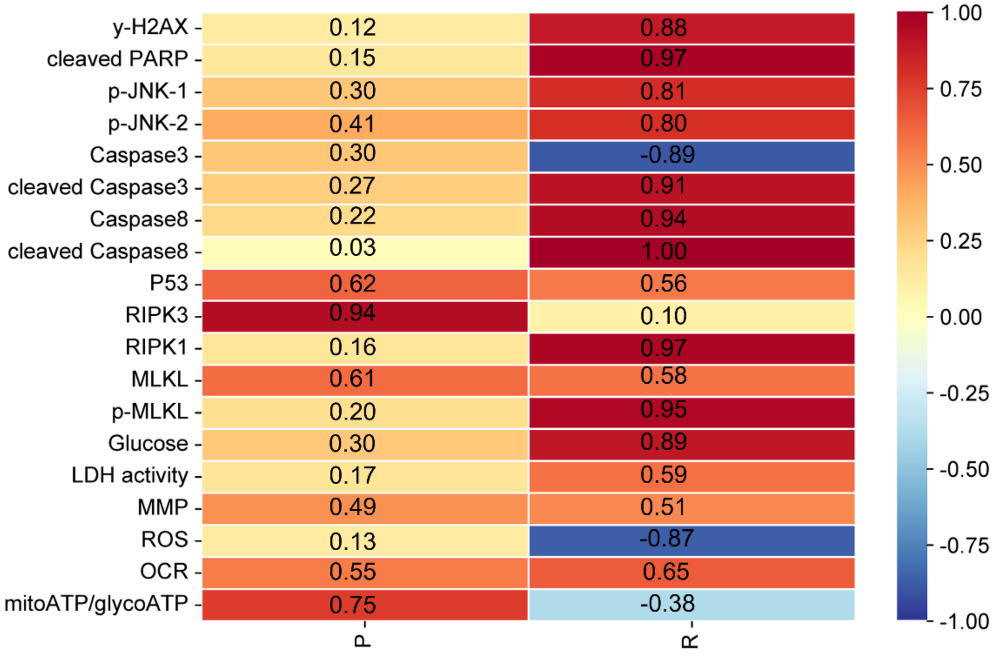

	P	R
y-H2AX	0.12	0.88
cleaved PARP	0.15	0.97
p-JNK-1	0.30	0.81
p-JNK-2	0.41	0.80
Caspase3	0.30	-0.89
cleaved Caspase3	0.27	0.91
Caspase8	0.22	0.94
cleaved Caspase8	0.03	1.00
P53	0.62	0.56
RIPK3	0.94	0.10
RIPK1	0.16	0.97
MLKL	0.61	0.58
p-MLKL	0.20	0.95
Glucose	0.30	0.89
LDH activity	0.17	0.59
MMP	0.49	0.51
ROS	0.13	-0.87
OCR	0.55	0.65
mitoATP/glycoATP	0.75	-0.38

Figure S8. Correlation analysis of the investigated parameters in GC-1 cells between the 1 uM DBDPE exposure group and the oxamate exposure group. For most of the investigated parameters were significantly changed in 1 μM DBDPE exposure group and oxamate exposure group, with similar change trends observed. A pearson correlation analysis were conducted to further figure out their changing correlationship. "*R*" and "*P*" indicate the correlation coefficient and the significant difference, respectively; their values are shown in the figure. Red and blue colour represent positive and negative correlation, respectively.

Supplementary Tables

Table S1. The measured sperm motility parameters and accompanying definitions in this study.

Parameters	Unit	Definition
Total motility (TM)	%	Percentage of sperms that are moving actively either linearly or in a large circle regardless of speed.
Progressive motility (PM)	%	Percentage of motile sperms that expressed vigorous swimming.
Average path velocity (VAP)	μm/s	The average point-to-point velocity of sperm along its average path.
Straight line velocity (VSL)	μm/s	The average point-to-point velocity along the straight line between its first and last detected position.
Curvilinear velocity (VCL)	μm/s	The average point-to-point (total distance traveled) velocity of sperm along its track.
Linearity (LIN)	%	The ratio of VSL/VCL describes path curvature. Percentage linearity of the sperm path, calculated by the departure of the actual sperm track from a straight line.

Table S2. CASA input parameters for zebrafish.

Input parameters	Input values	Unit
Minimum sperm head size	1	μm^2
Maximum sperm head size	100	μm^2
Minimum tail length	8	μm
pixel/μm ratio	151/100	-
Maximum tail length	15	μm
Minimum track length	10	frames
Maximum sperm velocity between frames	20	pixels/frame
Minimum VSL for motile	3	μm/s
Minimum VAP for motile	5	μm/s
Minimum VCL for motile	15	μm/s
Low VAP speed threshold	10	μm/s
Maximum percentage of the path with low VAP	25	unitless
High WOB, (percentage of VAP/VCL)	80	unitless
High LIN, (percentage of VSL/VAP)	80	unitless
Minimum frames per second	16	frames

Table S3. Detailed informations of primers for qRT-PCR used in the present study

Gene name	Full name	Primer	Acession number	product size (bp)
rpl8	ribosomal protein L8	F: TTGTTGGTGTTGTTGCTGGT R: GGATGCTCAACAGGGTTCAT	XM_005167684.1	136
caspase9	apoptosis-related cysteine peptidase	F: CTGGAAACTCTTGTCAGAATGG R: TTCAATGCCTTGACGAGGTTTA	NM_001007404.2	80
ripk3	receptor-interacting serine-threonine kinase 3	F: TTGTCCCGAGTGGCTGAAAA R: ACGGCTCCTTCCCAGTGATA	XM_001343791.5	181
parp1	poly (ADP-ribose) polymerase 1	F: GACTCACAGGACGACAAGC R: GACTTTGCCATCAAACATGGG	NM_001044942.1	141
parp2	poly (ADP-ribose) polymerase 2	F: CTTCAGAGGCGAAGACGGTT R: TCAAAGCATTCCCTTCATTCAGAT	NM_001204270.1	246
parp3	poly (ADP-ribose) polymerase family, member 3	F: AAGCTGCTTTGGCATGGAAC R: GTTTGATGGACGCACATAGCC	NM_200501.1	153
pold1	polymerase (DNA directed), delta 1, catalytic subunit	F: CTCCTCCCACTTCGTTCCAC R: CATATACGCCGCAACTCCCT	NM_001039810.2	466
pold3	polymerase (DNA-directed), delta 3, accessory subunit	F: AGTGCTATACGGTGTGCTGC R: TTGGAGGTAGCAGCAGGTTC	NM_001042769.2	134
lig3	ligase III, DNA, ATP-dependent	F: AGCAAAGGCTGCGAAAACAG R: CCTAGCGTGTGTGTGGCTAA	NM_001030174.2	300
rpa1	replication protein A1	F: CAGCTCAATCCCATGGCTGA R: GGTGCCTTTGACTGACCCTC	NM_199811.2	197
tp53bp1	tumor protein p53 binding protein, 1	F: CTAACCCTGTCGCATCCTTATG R: AGGCTGAGAGTCCTCAACTAT	NM_001080170.1	100
brca2	BRCA2 DNA repair associated	F: GGCTTGCTTACAGAGGGACA R: CAACCATTCTGGAGACACCTCA	NM 001110394.2	144
brat1	BRCA1-associated ATM activator 1	F: GATCAACTTGCTGGAGGAGGC R: CACTGCATACGGACAGCAGG	NM_001040305.1	259
mcm2	minichromosome maintenance complex component 2	F: CAGATGGACCAGGACAAAGT R: ACGCACAGTAATCGGGATA	NM_173257.2	87

msh6	mutS homolog 6	F: GGGAGGCAAATCCACTCTAATG R: GACTCGATCCACAGGAGTAAGA	NM_182860.1	109
cdk10	cyclin dependent kinase 10	F: TCACGGAGTTTCCACCTCCT R: TATCGCGTGCTCGGTACAC	NM_001017622.2	289
cdk12	cyclin dependent kinase 12	F: ACTGCAGGGACAGTCCAAAG R: CCAGTCGGTTTGAGTCTGCT	XM_003200531.5	263
cdk11b	cyclin dependent kinase 11B	F: CCCGCACGAAAGACAAAGAG R: GCTCCAGACGATCCCTCTCTC	NM_001008646.1	150
cdk13	cyclin dependent kinase 13	F: AGTGCTCCAACATCCTGCTC R: GCTGCCGCAGATACGACTTA	NM_001366829.1	281
jak3	Janus kinase 3	F: AACAGAGCGAGCAGCAGAGAG R: GTGTGACCACCCTTCCTTCC	XM_002663087.6	177
akt1s1	AKT1 substrate 1	F: TCGGCAGGTGTCTTCTCAAT R: ACCCATTGCCATACCACGAG	XM_687419.9	141
pik3r4	phosphoinositide-3-kinase, regulatory subunit 4	F: AAGAGAGCTCGCAAACATACGA R: CCACTGTCTGGAATCGTCGAG	XM_005158299.4	119
pik3c2a	phosphatidylinositol-4-phosphate 3-kinase, catalytic subunit type 2 alpha	F: CTCCAGCAGGAACTGACGTT R: TGTTTGCGCTGTTGGATTGG	XM_005159305.4	368
ndufc2	NADH:ubiquinone oxidoreductase subunit C2	F: TCCGCCTAAACATTGCCCTT R: GACGATGAACACCGGCTTTG	NM_001013517.1	239
uqcrc2b	ubiquinol-cytochrome c reductase core protein 2b	F: GACCTCACGGGAAGGGTGAA R: TCAGTGTGCTGGTGCTGCTG	NM_001001589.1	117
atp6v0e1	ATPase H+ transporting V0 subunit e1	F: TCCAGCGTCACATGATCGTC R: CATCGTCACAATCACCCCGC	NM_001006038.2	215
atp5l	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit g	F: TCGTGGCTAAAGTGCCAACA R: TCCTCACAGCATCCCTGACT	NM_199757.1	204
slc25a5	solute carrier family 25 member 5	F: CATCATTTACAGAGCTGCCTAC R: TTTACGTCCAGACTGCATCATC	NM_173247.1	184
coq5	coenzyme Q5, methyltransferase	F: ACCCACATCGAGCAAGCTTTA R: TACTGGACGCGGAAGAAACC	NM_001004541.1	266
coq6	coenzyme Q6 monooxygenase	F: CGACCAGGATTCCAGCACTT R: CCAAGCACCAAGACCACTCA	NM_001045404.1	241
apex2	APEX nuclease (apurinic/apyrimidinic endonuclease) 2	F: TGAACACCTCTCACAGACCC R: ATCCAGCCACTTTCTCCCAG	NM_200146.1	86

eno4	enolase 4	F: CCCGAGCCATAGCAAAGTGT R: CACCGCACACCATCTTTTCC	NM_001077547.1	299
pklr	pyruvate kinase L/R	F: AGAAACAGCCAAAGGACA R: ACGAGGACGATAACGAGA	NM_201289.1	253
pkma	pyruvate kinase M1/2a	F: GTCCGCACACAGCACATGATTG R: GGGTCACGGGTCAGAACAGA	NM_199333.1	101
adh8a	alcohol dehydrogenase 8a	F: CGAGTACACCGTCATCAAC R: AGCACCGAGTCCGAATAC	NM_001001946	166

Table S4. DBDPE contents detected in zebrafish testes.

Nominal cocentrations (nM)	Measured concentrations (ng/g lw)	
	Mean ± SEM	Median
0	< LOD	< LOD
0.1	870.84 ± 98.45	957.98
1	1284.2 ± 482.54	1197.51
10	1410.83 ± 29.09	1480.83
100	3252.30 ± 219.40	3288.56

Note: LOD, limit of detection.

Table S5. Basic developmental parameters of exposed male fish and F1 offspring larvae.

Parameters		DBDPE concentration (nM)				
		0	0.1	1	10	100
Exposed male fish	Body length (cm)	2.94 ± 0.02	2.89 ± 0.02	2.97 ± 0.02	2.97 ± 0.02	2.85 ± 0.02*
	Body weight (g)	0.38 ± 0.01	0.37 ± 0.01	0.41 ± 0.01*	0.40 ± 0.01	0.36 ± 0.01
	Condition factor (%)	1.50 ± 0.02	1.54 ± 0.02	1.53 ± 0.02	1.50 ± 0.02	1.58 ± 0.02*
	Gonado-somatic index (GSI, %)	0.88 ± 0.04	0.95 ± 0.04	0.98 ± 0.05	0.97 ± 0.03	1.02 ± 0.06
	Egg production (number/pair)	246.6 ± 51.3	349.8 ± 51.9	438.1 ± 48.3	365.9 ± 55.3	338.2 ± 53.5
F1 embryos	Fertilization rate (%)	91.33 ± 1.10	92.38 ± 2.71	91.21 ± 1.50	94.71 ± 0.81	94.79 ± 0.87
	Hatching rate (72 hpf, %)	99.81 ± 0.19	89.00 ± 4.74	81.81 ± 2.98*	96.69 ± 0.74	83.78 ± 2.14*
	Malformation rate (120 hpf, %)	0.14 ± 0.08	0.52 ± 0.23	0.13 ± 0.08	0.72 ± 0.29	0.33 ± 0.25
	Survival rate (120 hpf, %)	80.84 ± 1.97	86.75 ± 0.99	78.56 ± 1.62	81.00 ± 0.74	79.56 ± 1.78

Note: Data were represented as the means ± standard error of the mean (SEM). $*P < 0.05$ indicates significant differences between the exposure groups and the control group by one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) test.